PHYSIOLOGIA PLANTARUM 103: 193–200. 1998
Printed in Ireland—all rights reserved

ISSN 0031-9317

Yellowing and photosynthetic decline of barley primary leaves in response to atmospheric CO_2 enrichment

Richard C. Sicher

Sicher, R. C. 1998. Yellowing and photosynthetic decline of barley primary leaves in response to atmospheric CO_2 enrichment. – Physiol. Plant. 103: 193–200.

The photosynthetic response of barley (*Hordeum vulgare* L. cv. Brant) primary leaves was studied as a function of chlorosis induced by CO_2 enrichment. Leaf yellowing, measured as changes of chlorophyll *a* and *b*, was more extensive in controlled environments at elevated (680 ± 17 μl l^{-1}) than at ambient (380 ± 21 μl l^{-1}) CO_2. Stomatal conductance of primary leaves was decreased by growth in elevated CO_2 between 11 and 18 days after sowing (DAS) when measured at both 380 and 680 μl l^{-1} CO_2. Internal leaf CO_2 concentration (C_i) was also lower for elevated- compared to ambient-CO_2-grown primary leaves between 11 and 14 DAS. Results suggest that non-stomatal factors were responsible for the decreased photosynthetic rates of elevated- compared to ambient-CO_2-grown primary leaves 18 DAS. Various photochemical measurements, including quantum absorptance (α), minimal (F_0), maximal (F_m), and variable (F_v) chlorophyll fluorescence, as well as the F_v/F_m ratio, were significantly decreased 18 DAS in the elevated- compared to ambient-CO_2 treatment. Photochemical (q_P) and nonphotochemical (q_N) chlorophyll fluorescence quenching coefficients of 18-day-old primary leaves did not differ between CO_2 treatments. Photosynthetic electron transport rates of photosystem II were slightly lower for elevated- compared to ambient-CO_2-grown primary leaves 18 DAS. Concentrations of α-amino N (i.e. free amino acids) in barley primary leaves were increased by CO_2 enrichment 10 DAS, but subsequently, α-amino N decreased in association with photosynthetic decline. Total acid protease activity was greater in elevated- than in ambient-CO_2-grown leaves 18 DAS. The above findings suggest that photoinhibition and premature senescence were factors in the CO_2-dependent yellowing of barley primary leaves.

Key words – Barley, chlorophyll fluorescence, chlorosis, CO_2 enrichment, *Hordeum vulgare*, photosynthetic acclimation, premature senescence, quantum absorptance.

R. C. Sicher (e-mail rsicher@asrr.arsusda.gov), USDA, Agricultural Research Service, Climate Stress Laboratory, Beltsville Agricultural Research Center, 10300 Baltimore Avenue, Beltsville, MD 20705–2350, USA.

Introduction

Chlorosis and various other forms of leaf injury have been observed during plant growth in a CO_2-enriched atmosphere (Cave et al. 1981, Sasek et al. 1985, Wullschleger et al. 1992). Leaf yellowing usually became prevalent on plants grown at high compared to low photosynthetically active radiation (PAR) and was greater in mature than in younger leaves (Madsen 1974, Tripp et al. 1991a,b). This foliar damage adversely affected the growth and yield of glasshouse crops (Nederhoff and Buitelaar 1992, Nederhoff et al. 1992). Reduced plant growth due to leaf injury was attributed to decreased light interception and to decreased photosynthetic rates in response to CO_2 enrichment (DeLucia et al. 1985, Sasek et al. 1985). Betsche (1994) proposed several mechanisms that could explain the development of leaf injury and photosynthetic decline of cotton leaves exposed to elevated CO_2. These included photobleaching, photoinhibition, premature

Received 21 November, 1997; revised 9 March, 1998

senescence, inhibition of photosynthetic electron transport by HCO_3^- and inhibition of photosynthesis by inadequate rates of end product synthesis. It has also been suggested that leaf yellowing is the result of a nutrient insufficiency that develops during growth in elevated CO_2 (Chen and Lenz 1997, Keutgen et al. 1997).

In a previous study (Sicher 1997), leaf yellowing was observed on barley primary leaves between 9 and 17 days after sowing (DAS). Chlorosis occurred during growth at moderate to high PAR and was enhanced by CO_2 enrichment. It was concluded that photobleaching was likely involved in the development of chlorotic lesions on barley primary leaves. These findings were preliminary and additional studies are required to better understand the mechanisms responsible for leaf injury during growth at high PAR and CO_2.

Previous experiments with field-grown winter wheat suggested that leaf senescence was accelerated by CO_2 enrichment (Sionit et al. 1980, Nie et al. 1995, Sicher and Bunce 1997). This conclusion was based on the finding that decreased photosynthetic rates and the mobilization of chlorophyll (Chl) and soluble protein occurred earlier in wheat flag leaves grown at elevated compared to ambient CO_2. Previous studies with intact barley primary leaves grown in ambient CO_2 showed that changes of α-amino N and acid protease activity occurred during senescence (Friedrich and Huffaker 1980). Effects of CO_2 enrichment on changes of α-amino N and acid protease activity during senescence have not been investigated previously. The objective of the current study was to test the hypothesis that premature senescence contributed to chlorosis and photosynthetic decline of barley primary leaves grown in a CO_2-enriched atmosphere (Betsche 1994). It was also of interest to determine if leaf yellowing at elevated CO_2 involved photoinhibition.

Abbreviations – C_i, internal CO_2 concentration; DAS, days after sowing; ETR, photosynthetic electron transport rate; F_m, maximal fluorescence; F_0, minimal fluorescence; F_v, variable fluoresence; PAR, photosynthetically active radiation; q_N, nonphotochemical quenching; q_P, photochemical quenching; α, quantum absorptance; Y, fluorescence yield of photosystem II.

Materials and methods

Plant material

Barley (*Hordeum vulgare* L. cv. Brant) seedlings were grown at 22°C in controlled environment chambers (model M-2, Environmental Growth Chambers Corp., Chagrin Falls, OH, USA) using a 14/10 h light/dark cycle. Plants were grown at a PAR of 950 ± 30 μmol m^{-2} s^{-1} measured at pot level and this was provided by high pressure Na and metal halide lamps (Lucolux LU 400 W and Multivapor MVR 400 W, General Electric Co., Cleveland, OH, USA) located above a glass barrier. Plants were seeded in plastic pots containing 1.0 l of vermiculite and pots were watered daily with a complete mineral nutrient solution (Robinson 1984). Pots were thinned to single plants after 7 days of growth. Relative humidity was not controlled but was always greater than 50%. Chamber air CO_2 concentrations were controlled as described previously (Sicher 1997). Ambient and elevated CO_2 concentrations were 380 ± 21 and 680 ± 17 μl l^{-1} CO_2, respectively, based on 24-h means. Experiments were replicated in the same two chambers after reversing the ambient and elevated CO_2 concentrations.

Gas exchange measurements

Photosynthesis and transpiration rates of barley primary leaves were determined at 2- to 3-day intervals between 11 and 18 DAS. Net CO_2 exchange rates and stomatal conductances to water vapor were measured with a portable, open path infrared gas analysis system (CIRAS-1, PP Systems, Haverhill, MA, USA) equipped with CO_2 control. Photosynthetic rates of plants grown with ambient and elevated CO_2 were measured at both 380 and 680 μl l^{-1} CO_2 in the environmental chambers used for plant growth. The leaf cuvette was attached to a pair of primary leaves from two plants at midlength and PAR, temperature and humidity were similar to those in the growth chamber. Measurements were repeated until consecutive values varied by less than 5%. Net CO_2 exchange rates, stomatal conductances to water vapor and intercellular CO_2 concentrations (C_i) were calculated from the output of the gas exchange system (Bunce 1988). Values are expressed as means $\pm$SE for $n = 4$.

Leaf absorptances

Optical absorptivity values for single barley primary leaves were determined using an Ulbricht integrating sphere as described by Idle and Proctor (1983). A custom-made sphere having an internal surface area of 0.072 m^2 was freshly painted with integrating sphere coating (General Electric Co., Chemical Products Div., Cleveland, OH, USA). The light source (model LS-2, Hansatech Instr. Ltd, King's Lynn, UK) contained a 100-W Osram xenon lamp and diffuse PAR was detected at the sphere's surface with a quantum sensor located behind a light baffle (Lambda Instr., Lincoln, NE, USA). Quantum absorptance (α) was calculated from radiant flux measurements performed with and without a single detached primary leaf in the sphere. The inherent absorptivity of the sphere was determined with a standard absorber made of black matte paper. Leaf area was measured immediately after determining absorptivity with a leaf area meter (model 3000, Lambda Instr.). Samples were then frozen in liquid N_2

and saved for pigment analysis (see below). Absorptivity was expressed as means ± SE for n = 5.

Chlorophyll fluorescence

Chlorophyll fluorescence kinetics of barley primary leaves were measured with a modulated fluorometer (Opti-Sciences model 500, PP Systems, Haverhill, MA, USA). Two primary leaves were placed in a leaf clamp. The illumination and measurement signals were provided by a trifurcated fiber-optic light guide held at either a 90° or a 45° angle in proximity to the upper leaf surface. Fluorescence measurements were performed in the environmental chambers used for plant growth with temperature and CO_2 as described above. Actinic light at the leaf's surface was 700 μmol m^{-2} s^{-1} PAR because of shading by the light guide and leaf clamp. Minimal fluorescence (F_0), maximal fluorescence (F_m) and variable fluorescence (F_v) were determined prior to the start of the daily photoperiod using dark-acclimated leaves. Red modulated light and saturating flashes (0.8 s) of white light (3 000 μmol m^{-2} s^{-1} PAR) were provided by the fluorometer. Fluorescence ratios of light-acclimated leaves were used to determine the quenching coefficients q_P and q_N (Schreiber et al. 1986, Schindler and Lichtenthaler 1996). Photosynthetic electron transport rates (ETR) were calculated according to Genty et al. (1989) and absorptivity and PAR were as described above. Fluorescence measurements for each date and CO_2 treatment were expressed as means ± SE for n = 5.

Biochemical measurements

Free α-amino N was assayed by the ninhydrin procedure according to Friedrich and Huffaker (1980). Single barley primary leaves were homogenized in a ground glass tissue grinder at 0°C with 5 ml of 80% (v/v) ethanol. Extracts were centrifuged at 3 000 *g* and the pellets were discarded. Samples were diluted to an appropriate concentration with distilled H_2O and then mixed with three volumes of ninhydrin reagent. Color was developed for 20 min in a boiling water bath. Reaction mixtures were diluted with 4 volumes of 50% (v/v) *n*-propanol and absorbance was measured at 570 nm. Blanks were prepared without plant samples and standard curves were prepared with known amounts of glycine. An aliquot of each supernatant was also used to measure Chl *a*, Chl *b* and carotenoids (x + c) in 80% (v/v) acetone according to Lichtenthaler (1987).

Changes of proteolytic activity in barley primary leaves were determined with azocasein as substrate (Friedrich and Huffaker 1980). Single barley primary leaves were homogenized at 0°C in a ground glass tissue homogenizer with 3 ml extraction buffer containing 50 m*M* HEPES-NaOH (pH 7.5), 1 m*M* EDTA and 5 m*M* DTT. Samples were spun in an Eppendorf microcentrifuge (model 5415C) for 3 min at full line voltage and the resultant supernatants were quickly frozen in liquid N_2. Immediately after thawing, a 0.2-ml aliquot of each extract was mixed with 0.4 ml azocasein (10 mg ml^{-1}) and 0.4 ml citrate buffer (pH 5.2). Assays were terminated after 2 h at 38°C with 1 ml of 10% (v/v) TCA. Blanks of each sample were prepared by adding TCA to the assay mixture prior to the plant extracts. Precipitates were removed by centrifugation, as above, and the absorbance of each sample and blank was measured at 340 nm. Protease activity is reported as the net absorbance change (340 nm) per second corresponding to 1 m^2 leaf area. Protease measurements were expressed as means ± SE for n = 5.

Data analysis

Statistical differences in response to CO_2 treatment usually were determined at $P \leq 0.05$ using an unpaired Student's *t*-test. Gas exchange measurements were compared by date and by CO_2 treatment using a one-way analysis of variance procedure (SuperANOVA, Abacus Concepts, Berkeley, CA, USA) as described previously (Sicher and Bunce 1997).

Results

Leaf injury and photosynthetic decline

In the present study and previously (Sicher 1997), yellowing was observed on primary leaves of 2- to 3-week-old barley plants and chlorosis was more extensive on seedlings grown in elevated compared to ambient CO_2. Maximal Chl *a* concentrations (0.3 g m^{-2}) of barley primary leaves grown in both ambient and elevated CO_2 were observed 12 DAS (Fig. 1A). Primary leaf Chl *a* levels of ambient- and elevated-CO_2-grown plants were 40 and 62% below maximal levels, respectively, when measured 18 DAS. Levels of Chl *a*, Chl *b* and total carotenoids (x + c) decreased in barley primary leaves in response to leaf aging in both CO_2 treatments (Fig. 1B,C). The Chl *a*/*b* ratio of primary leaves of both ambient- and elevated-CO_2-grown seedlings was 4.8 ± 0.1 ($P \geq 0.05$), when averaged over all five measurement dates between 10 and 18 DAS. Primary leaf Chl *a* plus *b* levels of ambient- and elevated-CO_2-grown plants differed ($P \leq 0.05$) 16 and 18 DAS. Total carotenoid (x + c) levels in primary leaves of seedlings grown in elevated compared to ambient CO_2 were similar except on the last sampling date.

Gas exchange rates of barley primary leaves grown in ambient and elevated CO_2 were determined at both 380 and 680 μl l^{-1} CO_2. Stomatal conductance to water vapor was significantly lower ($P \leq 0.001$) for primary leaves grown in elevated compared to ambient CO_2 on all measurement dates and at both measurement CO_2

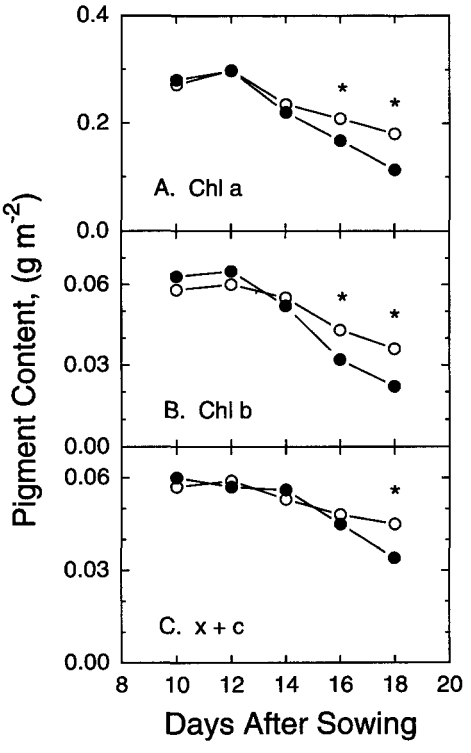

Fig. 1. Changes of Chl and carotenoid levels in barley primary leaves in response to elevated CO_2. Concentrations of Chl *a* (A), Chl *b* (B) and carotenoids (C) in barley primary leaves were measured at indicated times during growth at either 380 µl l^{-1} (○) or 680 µl l^{-1} (●) CO_2. An asterisk located above a symbol denotes a significant difference at $P \leq 0.05$.

concentrations. In general, stomatal conductance of both ambient- and elevated-CO_2-grown plants decreased with leaf age ($P \leq 0.05$). When combined over all measurement dates, internal CO_2 concentrations (C_i) of primary leaves grown in ambient CO_2 were 274 ± 4 and 571 ± 17 µl l^{-1} when measured in 380 and 680 µl l^{-1} CO_2, respectively. Comparable C_i measurements performed using primary leaves grown in elevated CO_2 were 237 ± 11 and 513 ± 30 µl l^{-1}, respectively ($P \leq 0.001$). In contrast to stomatal conductance, C_i determinations at both measurement CO_2 concentrations did not differ with leaf age ($P \geq 0.05$).

Net CO_2 fixation rates of primary leaves grown in both ambient and elevated CO_2 were maximal 11 DAS when measured in both 380 and 680 µl l^{-1} CO_2 (Fig. 2A,B). Rate measurements performed on subsequent dates decreased with leaf age. The effects of growth CO_2 concentrations on photosynthetic rates were significant ($P \leq 0.001$) for rate measurements performed at both 380 and 680 µl l^{-1} CO_2. When averaged over all measurement dates, net CO_2 fixation rates of primary leaves grown in elevated CO_2 and determined at 380 µl l^{-1} CO_2 were reduced 29.9% in comparison to the ambient CO_2 treatment. Similar comparisons of net CO_2 fixation rates measured at 680 µl l^{-1} CO_2 were 16.2% lower for primary leaves grown in elevated compared to ambient CO_2. Net CO_2 fixation rates of leaves from both CO_2 treatments measured at either 380 or 680 µl l^{-1} CO_2 also differed ($P \leq 0.001$). However, the difference between growth treatments by date was nonsignificant ($P \geq 0.05$) for CO_2 exchange rate measurements performed at 380 µl l^{-1} CO_2 but was significant ($P \leq 0.001$) for measurements performed at 680 µl l^{-1} CO_2.

Leaf absorptance and Chl fluorescence

Absorptance changes of barley primary leaves in response to leaf aging and CO_2 treatment were similar to changes of Chl concentrations described above. The optical absorptivity (α) of barley primary leaves grown in both ambient and elevated CO_2 was 0.83 ± 0.02 ($P \geq 0.05$) 10 DAS (Tab. 1). Subsequently, α decreased in mature leaves with visible symptoms of leaf injury. When measured 18 DAS, α of barley primary leaves grown in ambient and elevated CO_2 decreased 11 and 22% ($P \leq 0.05$), respectively.

Increasing the ambient CO_2 concentration had a negligible effect on Chl fluorescence and on fluorescence quenching of recently expanded 10-day-old barley

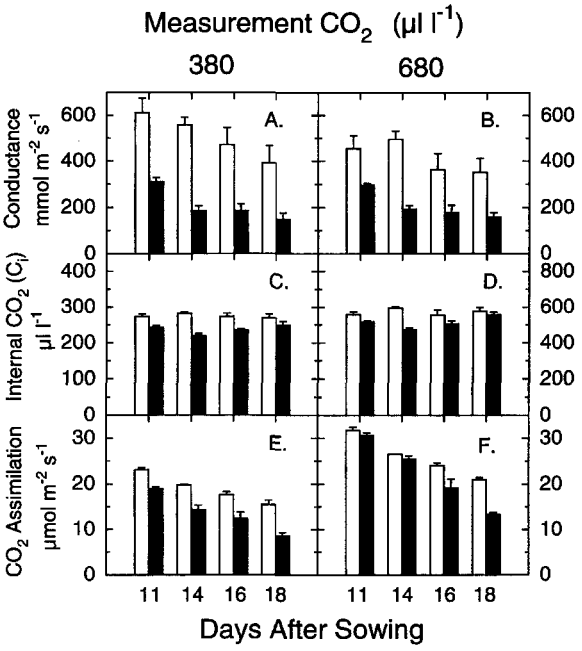

Fig. 2. Gas exchange properties of barley primary leaves during growth in ambient and elevated CO_2. Stomatal conductance (A,B), C_i, internal CO_2 (C,D) and net rates of CO_2 exchange (E,F) of ambient (white bars) and elevated (black bars) CO_2-grown barley primary leaves were measured at 380 (A,C,E) and at 680 (B,D,F) µl l^{-1} CO_2 on four dates between 11 and 18 DAS. Values are means (±SE) for n = 4.

Tab. 1. Changes of leaf absorptance, Chl fluorescence and fluorescence quenching of barley primary leaves grown at ambient (380 ± 21 µl l^{-1}) and elevated (680 ± 17 µl l^{-1}) CO_2. Fluorescence was measured with a pulse modulated fluorometer and F_0, F_m and F_v/F_m were measured on dark-acclimated leaves. All other parameters were measured with light-acclimated leaves (700 µmol m^{-2} s^{-1} PAR). Values are means ($n = 5$) and are in relative units, except for ETR (µmol electrons m^{-2} s^{-1}). NS, non-significant ($P \geq 0.05$); * ($P \leq 0.05$).

Parameter	10 DAS			18 DAS		
	Ambient	Elevated	P	Ambient	Elevated	P
α	0.83	0.83	NS	0.74	0.65	*
F_0	145	130	NS	130	112	*
F_m	770	768	NS	675	394	*
F_v/F_m	0.810	0.831	NS	0.798	0.712	*
q_P	0.860	0.834	NS	0.770	0.731	NS
q_N	0.465	0.459	NS	0.381	0.381	NS
Y	0.616	0.603	NS	0.560	0.522	*
ETR	178	176	NS	146	118	*

primary leaves when leaf injury was minimal (Tab. 1). Minimal fluorescence (F_0), maximal fluorescence (F_m) and the variable fluorescence to maximal fluorescence ratio (F_v/F_m) of dark-acclimated leaves grown in ambient or elevated CO_2 were not significantly different 10 DAS ($P \geq 0.05$). The photochemical and nonphotochemical fluorescence quenching coefficients, q_P and q_N, as well as the yield of photosystem II (Y) and the photosynthetic electron transport rate (ETR) also did not differ ($P \geq 0.05$) 10 DAS, when leaf yellowing was minimal. However, fluorescence measurements of older barley primary leaves were affected by CO_2 enrichment. When measured 18 DAS, F_0, F_m and the F_v/F_m ratio were significantly lower in primary leaves grown in elevated compared to ambient CO_2. There was no effect of growth in elevated CO_2 on q_P and q_N fluorescence quenching measured 18 DAS. However, fluorescence Y and the ETR, which was calculated from the measured values for PAR, Y and absorptance (see above), were lower 18 DAS in primary leaves grown in elevated compared to ambient CO_2.

α-Amino N and total protease activity

Total α-amino N was measured in both young and mature barley primary leaves to better assess the effects of CO_2 enrichment on leaf yellowing and photosynthetic decline. Total α-amino N was 13.7 ± 0.5 and 21.0 ± 0.8 mmol m^{-2} ($P \leq 0.05$) in primary leaves grown in ambient and elevated CO_2, respectively, when measured 10 DAS (Fig. 3). Total α-amino N of ambient- and elevated-CO_2-grown primary leaves decreased 18 and 48%, respectively, between the first and last harvest. α-Amino N levels decreased earlier in barley primary leaves grown in elevated compared to ambient CO_2.

Total acid protease activity in barley primary leaves, measured at pH 5.2 using azocasein as a substrate, was barely measurable in extracts of 10-day-old primary leaves from either CO_2 treatment (Fig. 4). Total proteolytic activity of primary leaves grown in ambient and elevated CO_2 increased 4- and 7-fold, respectively, in an almost linear manner between 10 and 18 DAS. Protease activity was unaffected ($P \geq 0.05$) by CO_2 treatment between 10 and 14 DAS. However, proteolytic activity was 42 and 26% greater ($P \leq 0.05$) in primary leaves grown in elevated compared to ambient CO_2 when measured 16 and 18 DAS, respectively. Whole leaf soluble protein concentrations measured 18 DAS differed in the two CO_2 treatments used in the present study (data not shown). In agreement with the above protease measurements, soluble protein levels in ambient- and elevated-CO_2-grown primary leaves were 3.9 ± 0.3 and 3.1 ± 0.1 g m^{-2} ($P \leq 0.05$), respectively, on the last sampling date.

Discussion

In agreement with prior reports (Madsen 1974, DeLucia et al. 1985, Sasek et al. 1985, Tripp et al. 1991a,b, Sicher 1997), chlorotic lesions on barley primary

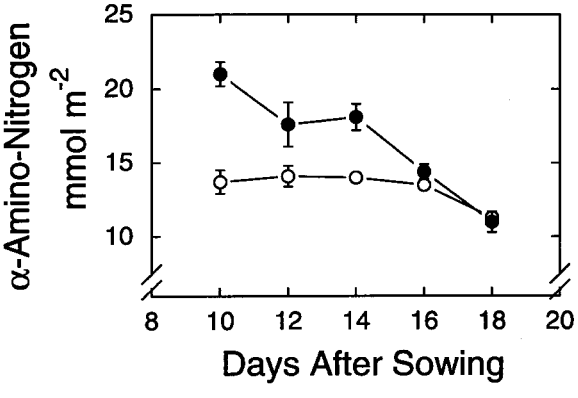

Fig. 3. Changes of α-amino N concentrations in barley primary leaves during growth in ambient (○) and elevated (●) CO_2. Experimental details were as in Fig. 1.

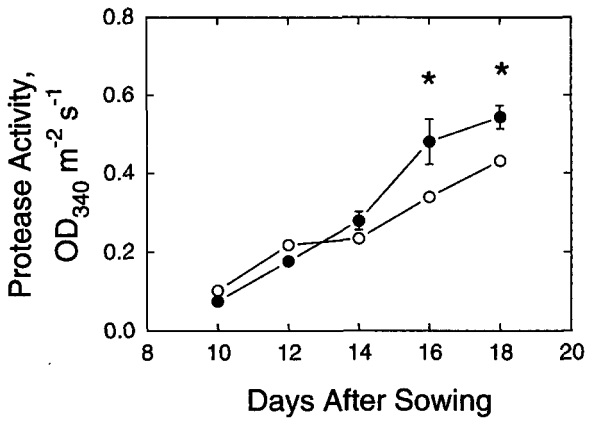

Fig. 4. Changes in proteolytic activity in barley primary leaves during growth in ambient (○) and elevated (●) CO_2. Acid protease activity was measured at pH 5.2 with azocasein as substrate. Other details were as in Fig. 1.

leaves were more extensive during growth in elevated than in ambient CO_2. Chlorosis and tip necrosis were the most common forms of leaf damage and these occurred between 2 and 3 weeks of growth. Pigment analyses supported the qualitative evidence of leaf yellowing discussed above. Leaf Chl concentrations decreased with age and the loss of Chl was greater at elevated than at ambient CO_2. Present findings also indicated that Chl *a* and *b* levels decreased more quickly than carotenoids. The Chl *a*/*b* ratio of barley primary leaves did not change between 10 and 18 DAS in either CO_2 concentration. Changes of the Chl *a*/*b* ratio in elevated-CO_2-grown strawberry plants with extensive chlorosis were correlated with damage to the photosynthetic reaction center core complexes (Keutgen et al. 1997). The relative changes in Chl *a* plus *b* and carotenoids in barley primary leaves in response to CO_2-dependent leaf injury were similar to pigment changes observed in senescing leaves (Woolhouse 1984).

Understanding the photosynthetic responses of plants to long-term (i.e. days to weeks) CO_2 enrichment is of widespread interest (Sage 1994). One approach used here was to compare net CO_2 exchange rates of plants grown and measured at ambient CO_2 with those of a similar population of plants grown and measured at elevated CO_2. As expected (Wulff and Strain 1982, Wullschleger et al. 1992), net CO_2 exchange rates of barley primary leaves grown in ambient and elevated CO_2 were substantially greater at 680 than at 380 μl l^{-1} CO_2 on all measurement dates. Thus, photosynthetic enhancement occurred at higher CO_2 concentrations in spite of the extensive leaf injury observed on mature leaves. Stomatal conductance to water vapor and C_i of barley primary leaves grown in elevated CO_2 were decreased relative to those measured on plants grown in ambient CO_2 on all measurement dates and at both measurement CO_2 concentrations. The reduction of C_i in primary leaves grown in elevated CO_2 was proportional to the decrease of net CO_2 exchange rates that was observed 11 and 14 DAS for rate measurements performed at 380 μl l^{-1} CO_2. Therefore, lower conductance probably accounted for the decreased CO_2 exchange rates of leaves grown in elevated compared to ambient CO_2 on those dates and at 380 μl l^{-1} measurement CO_2. This conclusion was supported by the finding that net CO_2 exchange rates of ambient- and elevated-CO_2-grown primary leaves were similar 11 and 14 DAS when measurements were performed at 680 μl l^{-1} CO_2. Large reductions of net CO_2 exchange rates were observed for primary leaves of elevated- compared to ambient-CO_2-grown seedlings 18 DAS, even though no substantial differences of C_i were detected at either measurement CO_2 concentration. Therefore, the decreased photosynthetic CO_2 fixation of elevated- compared to ambient-CO_2-grown leaves was probably not the result of stomatal factors on the last measurement date. This conclusion was consistent with the level of leaf injury observed during growth at elevated CO_2.

The α of recently expanded primary leaves was similar to values observed for other species (Ehleringer and Björkman 1977, Idle and Proctor 1983). In agreement with the extent of leaf yellowing, the α of elevated-CO_2-grown primary leaves 18 DAS was below the normal range. Changes of α due to losses of Chl *a* plus *b* and carotenoids during growth in elevated CO_2 could account for some of the decreased photosynthetic rates of older barley primary leaves exhibiting extensive leaf injury.

Primary leaves grown in elevated CO_2 emitted much less Chl fluorescence 18 DAS than leaves grown in ambient CO_2. The most significant effect of leaf yellowing on Chl fluorescence of primary leaves grown in a CO_2-enriched atmosphere was decreased F_m. Decreased Chl fluorescence of chlorotic barley primary leaves was not attributable to increased fluorescence quenching by q_P or q_N. These results are in contrast to findings showing that q_N increased markedly with leaf yellowing in cotton (Betsche 1994). Small differences in F_v/F_m between primary leaves grown in ambient and elevated CO_2 suggested that photoinhibition may have contributed to the formation of chlorotic lesions. However, it is not likely to be the primary factor responsible for leaf yellowing (cf. Krause and Weis 1991). Betsche (1994) similarly concluded that photoinhibition contributed little to leaf yellowing in cotton. Differences in fluorescence Y and ETR between ambient- and elevated-CO_2-grown primary leaves also were small. Therefore, the Chl fluorescence measurements reported here suggest that the integrity of photosystem II activity was only moderately affected by leaf yellowing.

Leaf senescence involves degradation of soluble proteins and total Chl and results in decreased photo-

synthetic rates (Friedrich and Huffaker 1980). In addition to Chl and soluble protein, changes of α-amino N have been observed in senescing cereal leaves (Wittenbach 1979, Friedrich and Huffaker 1980). α-Amino N of attached, ambient-CO_2-grown barley primary leaves decreased in older, senescing leaves (Friedrich and Huffaker 1980). In the present study, α-amino N initially increased in response to CO_2 enrichment. The age-dependent loss of α-amino N was proportionally larger and occurred earlier in primary leaves grown in elevated compared to ambient CO_2. Changes of α-amino N in barley primary leaves were closely correlated with the changes of photosynthesis reported above.

Proteolytic enzyme activity in cereal leaves increased during senescence and this occurred in conjunction with losses of soluble protein and Chl (Wittenbach 1979, Friedrich and Huffaker 1980). Multiple proteolytic enzymes are responsible for protein degradation in cereal leaves but endopeptidases and exopeptidases account for the bulk of the activity associated with senescence (Waters et al. 1980). Acid proteases are very likely localized in the vacuole and the role of these enzymes in senescence is uncertain (Woolhouse 1984). Proteolytic activity using azocasein as a substrate was greater in 16- to 18-day-old primary leaves grown in elevated compared to ambient CO_2. However, the increase of protease activity in response to CO_2 enrichment was small compared to that occurring with leaf age. In agreement with the acid protease measurements of the present study, soluble protein concentrations were 10 to 20% lower 18 DAS in primary leaves grown in elevated compared to ambient CO_2 (data not shown).

It was previously concluded that chlorosis of barley primary leaves at high PAR and elevated CO_2 resulted from losses of bulk Chl as a result of photobleaching (Sicher 1997). Present data based on gas exchange and fluorescence measurements generally supported this earlier conclusion. However, leaf yellowing is a complex biochemical and biophysical process. The CO_2-dependent yellowing of barley primary leaves involved a decrease in photosynthesis, Chl, soluble protein and α-amino N levels and an increase in acid protease activity. All of these factors have been previously associated with developmentally induced senescence (Friedrich and Huffaker 1980). However, in natural senescence decreases of soluble protein are large relative to Chl degradation (Woolhouse 1984). The opposite situation was observed in barley primary leaves both here and previously (Sicher 1997). Therefore, in addition to photobleaching, photoinhibition and premature senescence may have contributed to the CO_2-dependent yellowing of barley primary leaves.

Acknowledgments – J. A. Bunce kindly performed the gas exchange analyses and contributed interpretive comments on these results. F. Caulfield competently operated the controlled environment chambers and assisted with growing the plants. Mention of a trademark, proprietary product or vendor neither implies an endorsement nor constitutes a warranty of the product by the USDA.

References

Betsche, T. 1994. Atmospheric CO_2 enrichment: Kinetics of chlorophyll fluorescence and photosynthetic CO_2 uptake in individual, attached cotton leaves. – Environ. Exp. Bot. 34: 75–86.

Bunce, J. A. 1988. Mutual shading and the photosynthetic capacity of exposed leaves of field grown soybeans. – Photosynth. Res. 15: 75–83.

Cave, G., Tolley, L. C. & Strain, B. R. 1981. Effect of carbon dioxide enrichment on chlorophyll content, starch content and starch grain structure in *Trifolium subterraneum* leaves. – Physiol. Plant. 51: 171–174.

Chen, K. & Lenz, F. 1997. Responses of strawberry to doubled CO_2 concentration and phosphorous deficiency. I. Distribution of dry matter, macronutrients and carbohydrates. – Gartenbauwissenschaft 63: 30–37.

DeLucia, E. H., Sasek, T. W. & Strain, B. R. 1985. Photosynthetic inhibition after long-term exposure to elevated levels of atmospheric carbon dioxide. – Photosynth. Res. 7: 175–184.

Ehleringer, J. & Björkman, O. 1977. Quantum yields for CO_2 uptake in C_3 and C_4 plants. Dependence on temperature, CO_2 and O_2 concentration. – Plant Physiol. 59: 86–90.

Friedrich, J. W. & Huffaker, R. C. 1980. Photosynthesis, leaf resistances and ribulose-1,5-bisphosphate carboxylase degradation in senescing barley leaves. – Plant Physiol. 65: 1103–1107.

Genty, B., Briantais, J.-M. & Baker, N. R. 1989. The relationship between quantum yield of photosynthetic electron transport and quenching of chlorophyll fluorescence. – Biochim. Biophys. Acta 990: 87–92.

Idle, D. B. & Proctor, C. W. 1983. An integrating sphere leaf chamber. – Plant Cell Environ. 6: 437–439.

Keutgen, N., Chen, K. & Lenz, F. 1997. Responses of strawberry leaf photosynthesis, chlorophyll fluorescence and macronutrient contents to elevated CO_2. – J. Plant Physiol. 150: 395–400.

Krause, G. H. & Weis, E. 1991. Chlorophyll fluorescence and photosynthesis: The basics. – Annu. Rev. Plant Physiol. Plant Mol. Biol. 42: 313–349.

Lichtenthaler, H. K. 1987. Chlorophylls and carotenoids: Pigments of photosynthetic biomembranes. – Methods Enzymol. 148: 350–382.

Madsen, E. 1974. Effect of CO_2-concentration on growth and fruit production of tomato plants. – Acta Agric. Scand. 24: 242–246.

Nederhoff, E. M. & Buitelaar, R. 1992. Effects of CO_2 on greenhouse grown eggplant (*Solanum melongea* L.). II. Leaf tip chlorosis and fruit production. – J. Hortic. Sci. 67: 805–812.

– , Koning, A. N. M. & Rijsdijk, A. A. 1992. Leaf deformation and fruit production of glasshouse grown tomato (*Lycopersicon esculentum* [Mill.]) as affected by CO_2, planting density and pruning. – J. Hortic. Sci. 67: 411–420.

Nie, G. Y., Long, S. P., Garcia, R. L., Kimball, B. A., Lamorte, R. L., Pinter, P. J., Wall, G. W. & Webber, A. N. 1995. Effects of free-air CO_2 enrichment on the development of the photosynthetic apparatus in wheat, as indicated by changes in leaf proteins. – Plant Cell Environ. 18: 855–864.

Robinson, J. M. 1984. Photosynthetic carbon metabolism in leaves and isolated chloroplasts from spinach grown under short and intermediate photosynthetic periods. – Plant Physiol. 75: 397–409.

Sage, R. F. 1994. Acclimation of photosynthesis to increasing atmospheric CO_2: The gas exchange perspective. – Photosynth. Res. 39: 351–368.

Sasek, T. W., DeLucia, E. H. & Strain, B. R. 1985. Reversibility of photosynthetic inhibition in cotton after long-term exposure to elevated CO_2. – Plant Physiol. 78: 619–622.
Schindler, C. & Lichtenthaler, H. K. 1996. Photosynthetic CO_2-assimilation, chlorophyll fluorescence and zeaxanthin accumulation in field grown maple trees in the course of a sunny and a cloudy day. – J. Plant Physiol. 148: 399–412.
Schreiber, U., Schliwa, U. & Bilger, W. 1986. Continuous recording of photochemical and non-photochemical chlorophyll fluorescence quenching with a new type of modulation fluorometer. – Photosynth. Res. 10: 51–62.
Sicher, R. C. 1997. Irradiance and spectral quality affect chlorosis of barley primary leaves during growth in elevated carbon dioxide. – Int. J. Plant Physiol. 158: 602–607.
– & Bunce, J. A. 1997. Relationship of photosynthetic acclimation to changes of Rubisco activity in field-grown winter wheat and barley during growth in elevated carbon dioxide. – Photosynth. Res. 52: 27–38.
Sionit, N., Hellmers, H. & Strain, B. R. 1980. Growth and yield of wheat under CO_2 enrichment and water stress. – Crop Sci. 20: 687–690.
Tripp, K. E., Peet, M. M., Pharr, D. M., Willits, D. H. & Nelson, P. V. 1991a. CO_2-enhanced yield and foliar deformation among tomato genotypes in elevated CO_2 environments. – Plant Physiol. 96: 713–719.
– , Peet, M. M., Willits, D. H. & Pharr, D. M. 1991b. CO_2-foliar deformation of tomato: Relationship to foliar starch concentration. – J. Am. Soc. Hortic. Sci. 116: 876–880.
Waters, S. P., Peoples, M., Simpson, R. J. & Dalling, M. J. 1980. Nitrogen redistribution during grain growth in wheat (*Triticum aestivum* L.). – Planta 148: 422–428.
Wittenbach, V. A. 1979. Ribulose bisphosphate carboxylase and proteolytic activity in wheat leaves from anthesis through senescence. – Plant Physiol. 64: 884–887.
Woolhouse, H. W. 1984. The biochemistry and regulation of senescence in chloroplasts. – Can. J. Bot. 62: 2934–2942.
Wulff, R. D. & Strain, B. R. 1982. Effects of CO_2 enrichment on growth and photosynthesis in *Desmodium paniculatum*. – Can. J. Bot. 60: 1084–1091.
Wullschleger, S. D., Norby, R. J. & Hendrix, D. L. 1992. Carbon exchange rates, chlorophyll content, and carbohydrate status of two forest tree species exposed to carbon dioxide enrichment. – Tree Physiol. 10: 21–31.

Edited by A. J. Stemler